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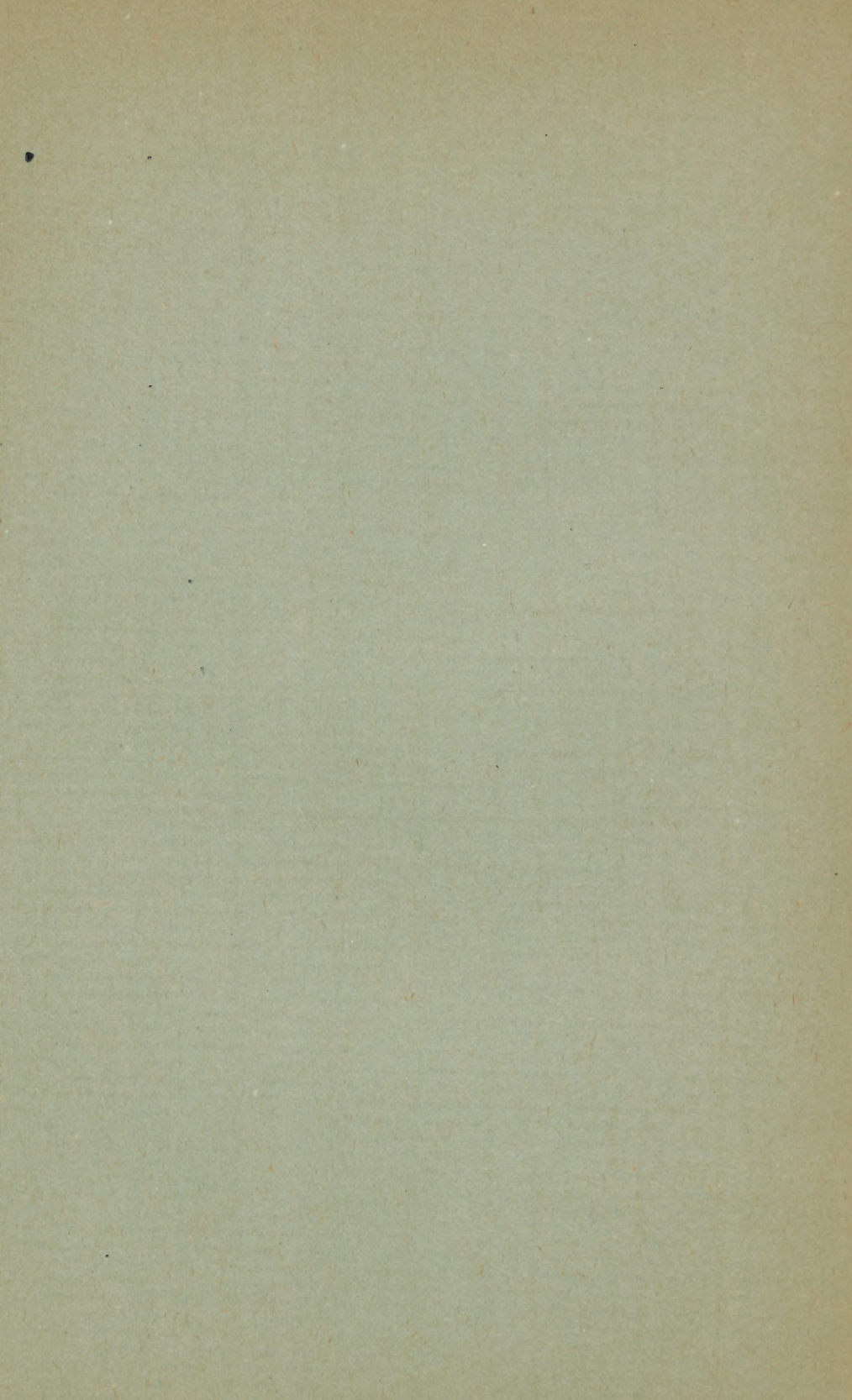
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Pathology, Johns Hopkins University.*

*[From the Pathological Laboratory of the Johns Hopkins University  
and Hospital.]*

(PRELIMINARY COMMUNICATION.)

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## BACILLUS PYOGENES FILIFORMIS (NOV. SPEC.).\*

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*Resident Pathologist, The Johns Hopkins Hospital; Associate Professor of Pathology, Johns Hopkins University.*

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During the past winter a large healthy female rabbit of the stock of the laboratory gave birth to a litter of young, and about the fifth day following parturition, although it had not appeared ill, was found dead in its cage. Following the rule of the laboratory, which is to make autopsies upon all animals which die, this one was examined in the usual way. It is necessary to state that the young of this animal were found dead before the death of the mother occurred.

The animal bore its litter on March 13th or 14th, and was found dead on the morning of the 18th. The autopsy was performed in the afternoon of the 18th.

The body was well nourished; there was no evidence of death from violence, the mammary glands were still large, and upon section a lactiferous fluid escaped from their cut surfaces. There was no excess of fluid in the peritoneal cavity, the layers of the serosa appearing normal except as is about to be mentioned.

The condition of the uterus especially arrested attention upon the examination of the abdominal viscera. It was several times larger than the normal, although much smaller than the uterus of the rabbit at term, and presented a series of dilatations and contractions which, except for their

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irregular distribution, might have been mistaken for a pregnancy. This condition was, however, hardly to be considered under the circumstances, and indeed upon inspection the dilated pouches appeared thin and semi-translucent, and gave the impression of being quite empty. The serosa over the dilatations was injected; the vessels of larger size being very prominent and turgidly filled with blood, the intervening tissue presenting a rosy hue. Both cornua of the uterus were similarly affected. Nothing abnormal was observed in connection with the ovaries. On opening the uterus after its removal with the vagina attached, the pallor of the mucous membrane contrasted with the injection of the serous coat. This pallor of the mucosa was of a peculiar opaque color and unlike the appearance of the velvety membrane itself. On gently stroking the mucosa with a knife a thick, opaque material could be removed, which appeared to be only lightly adherent to the surface of the membrane. It was to the presence of this material that the peculiar opacity was due, and the exudate extended from the vagina throughout the entire extent of the uterus. In the dilatations before mentioned the mucous membrane was of extreme tenuity, and here, as might have been expected, the layer of opaque exudate was thinner than elsewhere. The impression was given that the dilatations were brought about by accumulations of a gas. After the removal of the exudate the underlying mucosa was found to be congested. The other organs of the peritoneal cavity apparently were normal.

The pleural cavities contained fluid which was not in large amount and of a transparent hæmoglobin-red color. The serosa itself was covered with a thick shaggy layer of a fibrin-like material. Both the parietal and visceral layers were covered with this material, which was very thick over the region of the diaphragm. The lungs were in part voluminous, in part collapsed, the expanded portions being of a firm consistence and firmly consolidated. The bronchi contained fibrinous plugs.

The pericardial sac contained a small amount of fluid between layers of a shaggy appearance, due to the presence of a fibrinous-looking exudate similar to that found covering the pleuræ. It was, however, thinner here than there.



The lymph glands of the body showed no especial enlargement and the other viscera no gross pathological changes.

*Bacteriological Examination.* Cover-slips made from the exudate in the vagina and uterus showed a surprisingly large number of organisms which were strikingly pleomorphic. These organisms form for the most part thread-like structures; not a few, however, are much smaller. They vary from bits only a little larger than cocci to thread-like forms as long as the longest anthrax chains. At first sight there would appear to be several kinds of bacilli present; but the appearance, in both large and small forms, of a striking irregularity of staining makes this improbable. Very few of the bacilli stain regularly, for the most part brightly staining spots appearing between unstained areas. An outer membrane always stains, enclosing the stained dots in a colorless ground. A closer study reveals the stained particles to occur with much regularity, that is, they are about equidistant in the longer forms, where they are best studied, and in general they are of the same size. The threads are not as a rule straight, but present delicate sinuous and wavy outlines. The short forms are straight with rounded ends. Among these organisms a large number of pus cells and a few larger cells with single vesicular nuclei were scattered. Although many pus cells were present in the exudate, yet from the appearance of the cover-slips no inconsiderable portion of it must have been furnished by the bacilli.

Cover-slips made from the pericardial and pleural exudates, as well as from the consolidated portions of the lungs, showed the same organisms. While they were very numerous in the cover-slips from these situations, they were not as abundant as in the uterus.

Aerobic cultures were made upon various media, Loeffler's blood serum, sugar-agar, sugar-bouillon, plain agar and bouillon, the agar, urine and serum mixture; anaerobic cultures were made in plain and sugar-agar and bouillon as well as upon blood serum in Buchner's jars and an atmosphere of hydrogen. All these kept in the thermostat for several days at 37° C. showed no growth whatever.

Fearing that it would not be possible to cultivate the organism upon the usual media, the pleural cavity of a second rabbit

was inoculated by breaking up a speck of the pleural exudate from the first one in bouillon and injecting the suspension with a sterilized syringe, after making a small skin incision, into this cavity. This inoculation was positive in its results.

Subsequent experiments were conducted as in this one by transplanting small bits of the fibrinous material from the inflamed parts of previous animals, or of the fluid which was also present in the pleural cavities in the other animals. In this way the series was kept continuous and the bacilli alive.

Considerable variations were observed according as the inoculations were made into the pleural cavity, the peritoneal cavity, the subcutaneous tissues, beneath the dura mater, or directly into the circulation.

The inoculations were positive in all cases except a few in which they were made subcutaneously. The death of the animal occurred soonest when inoculation was made beneath the dura mater. A small portion of the skull was trephined, under the usual antiseptic precautions, and a drop of the pleural fluid or a speck of the fibrinous exudate was introduced beneath this membrane, care being taken not to injure the brain. These animals, which quickly recovered from the effects of the operation, died on an average about twelve hours after the inoculation. The usual appearances were as follows: The external layer of the dura, excepting at the point of puncture, appeared quite normal; the internal layer was injected. Corresponding with the area of congestion, but smaller, a grayish-white area was visible, this being most marked in the case of the introduction of a bit of the fibrin, and doubtless consisted in part of the introduced exudate. The pia was distinctly reddened, the vessels being more prominent than normal, and the meshes of the pia contained a thin but otherwise distinctly turbid fluid. There were no pathological changes to be observed in the cortex of the brain, nor were any found in the ventricles.

Cover-slip preparations made from the point of inoculation showed, besides pus cells, a very large number of the typical bacilli. Similar preparations from the meninges at a distance from the point of inoculation also showed bacilli, but they were fewer in number, and among them more or less leucocytes



with amphophilic granulations and polymorphous nuclei were scattered.

The pleural inoculations were followed by death, as before stated, in every instance, the death of the animal occurring upon the third or fourth day. The appearances presented at autopsy were for the most part an exact reproduction of those observed in the animal which had succumbed to the natural disease. Upon the side of inoculation a thick grayish-yellow shaggy membrane covered the pleural surfaces, being at times four or five millimeters in thickness. The pleural cavity contained several cubic centimeters of a clear hæmoglobin-colored fluid besides, the lung for the most part being compressed. At times smaller or larger areas of lobular pneumonia would be present; and as a rule the inflammation was not limited to the serous membrane of the side of inoculation, but extended into the opposite pleural cavity and into the pericardial sac. However, in these situations the process was as a rule less intense, the solid exudate being less considerable, and in the case of the opposite pleural cavity sometimes entirely wanting. The superficial vessels, however, were injected and the serous surfaces of the affected membrane covered with a slimy material. In addition to this, the opposite pleural cavity always contained a pink serum similar to that described upon the side of inoculation.

The study of the exudate upon the side of inoculation, as well as the fluid contained in the opposite pleural cavity and in the pericardium, showed the same organisms as had been introduced. They were most numerous upon the side of inoculation and in the solid portion of the exudate. So far as could be determined by the use of cover-slip preparations, they were absent from the blood and distant viscera.

The inoculation of the fluid from one of these pleural cavities into the peritoneum did not always succeed in causing the death of the animal. The periods of incubation in these cases, even when the inoculations were successful, were longer than in the previous ones, the animals affected often not dying in less than a week. The results of the peritoneal inoculations were to produce either a general sero-fibrinous peritonitis or a circumscribed fibrinous peritonitis. In several instances where the inoculations were made into the pleural

cavity, an extension through the diaphragm with the productions of a localized pseudo-membranous inflammation over the liver was observed. The exudate in all these cases showed large numbers of typical bacilli upon microscopical examination.

In several instances the subcutaneous inoculation of the pleural fluid was successful. Larger and smaller areas of tissue were converted into a rigid fibrinous material in which bacilli were found in large numbers.

Perhaps the most interesting, certainly the most widespread, effects were obtained by the intravenous inoculation of the pleural fluid. The results were uniformly fatal, the animals all succumbing in from two to four days after inoculation. At autopsy abscesses were present in the viscera. These were generally miliary in size, although at times they were larger and spreading. Preferences were exhibited in reference to their localization, certain organs being entirely spared. The abscesses were never absent from the brain and heart muscle. They appeared occasionally in the liver, more rarely still in the voluntary muscles, never in the kidneys or the lungs.

The effects of the intravenous inoculations with respect to the points of localization of the bacilli were in part determined by the local conditions; for example, the inoculation into non-pregnant female animals was not followed by the reappearance of the micro-organisms in any of the structures of the genital tract, whereas in the case of pregnant animals the inoculations were followed by the re-localization of the organisms and the inflammatory process in the pregnant uterus.

The appearances first described in the uterus of the animal dead of the natural disease indicated that an accumulation of gas had occurred in this structure. This appearance was again observed in the experimental disease in this situation, and also in several instances in which the inoculations were made into the subcutaneous tissue and in the pleural cavity, in the last instance the gas bubbles appearing in the inflamed mediastinal tissues.

*Cultivation Experiments.* From time to time in the course of the transference of this organism from animal to animal, attempts were made to cultivate it. The repeated use of ordinary media in aerobic and anaerobic cultures failed as in



the first instance. The use of more concentrated media, as for example five per cent. peptone in solid and fluid forms, also was without success. An attempt was now made to cultivate the organism upon the organs of a healthy rabbit, which were removed with all precautions and transferred to sterile test tubes. Only occasionally did one of these tubes show contamination. Those which were uncontaminated and had shown no growth for several days were inoculated with material from the experimental animals. For the first time a growth was obtained, not, however, upon all the organs. The growth was fairly vigorous upon the lungs, the heart and the uterus, less so upon the kidney; no growth occurred either upon the spleen or liver. Transplantations from these growths were successful only to the extent of one or two subsequent generations. The best results were obtained by cultivating the organisms upon several one-third to one-half grown foetuses obtained from the rabbit, upon which medium transplantations were successful through a series of six of these objects. The inoculations of animals from the sixth generation of the bacilli obtained in this way, either into the pleural cavity or into the circulation, were followed by positive results indistinguishable from those obtained by the use of the pleural fluid before mentioned.

Further facts concerning the morphology and biology of this organism, such as the question of spore formation, thermal death point, the effect of drying, the length of vitality outside the body, as well as the pathological histology of the lesions caused by it, will be given when the full details of this study are published.







